UNIVERSITI TEKNOLOGI MARA

LACTIC ACID BACTERIA (LAB) FERMENTED SOYMILK EXERTED ANTI-INFLAMMATORY EFFECTS VIA SUPPRESSION OF INFLAMMATORY RELATED CYTOKINES AND GENES IN DEXTRAN SULFATE SODIUM (DSS)-INDUCED COLITIS MICE

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Thesis submitted in fulfillment
of the requirements for the degree of
Master of Science

Faculty of Pharmacy

December 2014

AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledge as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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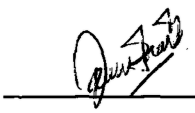
Signature of Student	:	
Date	:	December 2014

ABSTRACT

Ulcerative colitis (UC), a form of inflammatory bowel disease (IBD), is characterised by chronic inflammation of the colon. Its pathogenesis is associated with abnormal composition of the gut microflora. The use of probiotics, especially lactic acid bacteria (LAB), to improve gut microflora could thus serve as a good strategy in modulating inflammatory response during treatment of UC. As such, the principal aim of the present study was to identify LAB with potential anti-inflammatory effects against UC. For this purpose, 12 LAB isolated from Malaysian fermented food and dairy products were screened for their inhibitory effects against nitric oxide (NO), a known biomarker of UC using the Griess assay *in vitro*. The RAW 264.7 macrophages were cultured and served as the source of NO. Two LAB strains which elicited the most potent anti-NO activity, *Pediococcus pentosaceus* (LAB 8) and *Lactobacillus plantarum* (LAB 12), were then fermented in soymilk for validation of their anti-inflammatory effect *in vivo*. Colitis was induced in BALB/c mice (n = 12/ group) by administrating 3% DSS to drinking water for 7 days together with the LAB (10^9 cfu/200 µl). *Lactobacillus casei* strain Shirota (LABPC) and sulfasalazine were used as reference strain and positive control, respectively. After 28 days of pre-treatment with SM-LAB followed by 7 days of colitis induction, colons were removed for morphological examination before homogenised and being subjected to examination of gene expression (iNOS and NOD2) and physiological responses related to inflammation (NO, myeloperoxidase (MPO), lipid peroxidation and cytokines [tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin 17A (IL-17A), interleukin 6 (IL-6), and interleukin 12 (IL-12)] levels). Preliminary *in vitro* screening found LAB 8 and LAB 12 to exhibit significant ($p<0.05$) NO inhibitory effect when compared to the rest of the LAB and the constitutive NO synthase (NOS) inhibitor, N-nitro-L-arginine methyl ester (L-NAME) which served as positive control. Subsequent *in vivo* study using mice with DSS-induced colitis confirmed the anti-inflammatory effects of SM-LAB8 and SM-LAB12. The ability of SM-LAB8 and SM-LAB12 in reversing inflammation could be clearly observed morphologically from the histological analysis. Even though all groups administered by SM-LAB showed symptoms of less severe colitis as indicated by the percentage of weight loss, colon length and DAI scoring, only SM-LAB12 exhibited significant results ($p<0.05$). Furthermore, SM-LAB12 was also significantly ($p<0.05$) reduced concentrations of tested inflammatory markers which include NO, MPO, lipid peroxidation, TNF-α, IFN-γ, IL-6, IL-12, and IL-17A. Generally, the anti-inflammatory outcomes resulted by SM-LAB (except lipid peroxidation) were better as opposed to SM-LABPC. Both SM-LAB8 and SM-LAB12 also demonstrated their ability in significantly ($p<0.05$) suppressing IBD associated genes, iNOS and NOD2 in the colitis mice model. In conclusion, the results suggest that consumption of LAB strains is able to inhibit several inflammatory markers and alleviates UC. Although LAB may offer as new therapeutic option, further experiments must be conducted to understand the exact mechanisms behind their action.

ACKNOWLEDGEMENTS

It is an honour for me to be at this stage where I can express my gratitude to all who have assisted me in completing this study. Above all, praises to Allah for His blessings, guidance and for granting me the strength, patience and perseverance throughout the journey of completing this study.

This thesis would not have been possible without the help, support and patience of my principle supervisor, Assoc Prof Dr Kalavathy Ramasamy from the beginning until the very last stage of its completion, for which I am extremely grateful. She taught me not only on how to be a good student or researcher, but more importantly how to be a good supervisor. I would also like to express a great appreciation to my co-supervisor, Assoc Prof Dr Vasudevan Mani, for his assistance, valuable suggestions and time spent for me.

A very special and sincere thanks to all CDDR Lab members, especially Dr Lim Siong Meng, Azidah, Syafiqah, Adlia, Amalina, Aisyah, Nadia, Huda, Kathleen, Deena, Fareez, Zaki and all the juniors, for the friendship, assistance and for making this journey an enjoyable and memorable one. Not to forget, Brain Lab members; especially Murnirah, Noor and Shida, Pharmaco Lab members; Rosaimawati, Norhafizal, Siti Sarah, and Farhana, LAFAM staffs, and also Prof Mahmood from Faculty of Medicine, UM for their assistance and knowledge shared.

I appreciate the opportunity and financial support provided by International Islamic University of Malaysia and Ministry of Higher Education.

I am extremely grateful to all my family members, in laws and akhawats for their understanding, encouragement and prayers.

Last but not least, I would like to express my deepest gratitude to my beloved husband, Abu Fatih for always being by my side throughout the struggle and hardship, and my kids Fatih, Hamzah and Farouq for making me smile even during the hard times.

To those who indirectly contributed in this study, your kindness means a lot to me. Thank you.

TABLE OF CONTENTS